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# Evaluation of Combining Upper Respiratory Tract Swab Samples With Cerebrospinal Fluid Examination for the Diagnosis of Enteroviral Meningitis in Children

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In a prospective comparative study, the use of combined analysis of upper respiratory tract swab samples and cerebrospinal fluid (CSF) samples was assessed to improve the detection rate of enteroviral meningitis in children. An enterovirus was detected in 32% of patients with aseptic meningitis when testing CSF samples alone compared with 71.5% when combining CSF and respiratory tract findings. An enterovirus was detected in 17% of respiratory tract samples in an age- and sex-matched control group without meningitis. Thus, combining the examination of upper respiratory tract with CSF findings may improve the detection rate of enteroviral meningitis. Upper respiratory tract samples should be included in the diagnosis scheme to differentiate benign enteroviral meningitis from other life-threatening infections of the central nervous system. ***J. Med. Virol. 57: 193–197, 1999.*** © 1999 Wiley-Liss, Inc.

**KEY WORDS:** aseptic meningitis; enterovirus; cerebrospinal fluid; pharyngeal swabs; polymerase chain reaction

## INTRODUCTION

Aseptic meningitis is a common illness among children [Rantakallio et al., 1986; Wilfert et al., 1983]. Its etiologic agent is primarily nonpolio enteroviruses followed by mumps virus, although the prevalence of the latter organism is diminishing due to vaccination [Chonmaitree et al., 1989]. Seasonal outbreaks of enterovirus (EV) meningitis have been reported widely [McIntyre and Keen, 1993; Rantakallio et al., 1986; Rice et al., 1994]. In children, this disease is generally benign and requires neither specific therapy nor prolonged hospitalization, except in neonates and immunocompromized hosts [Haddad et al., 1993; Rorabaugh et al., 1993; Rotbart, 1995a; Sun et al., 1993]. The presentation of some atypical cases of EV meningitis may mimic bacterial meningitis or herpes encephalitis, emphasizing the need for rapid and accurate diagnosis to differentiate these infections clearly [Rotbart, 1995b]. Specific diagnosis of meningitis has relied on the detection of a specific pathogen in a cerebrospinal fluid (CSF) sample [Chonmaitree et al., 1989; Rotbart, 1990; Schlesinger et al., 1994; Wilfert et al., 1983; Yerly et al., 1996]. Viral detection in CSF can be accomplished by cell culture or by polymerase chain reaction (PCR), the latter being more sensitive [Lina et al., 1996; Yerly et al., 1996]. Despite the sensitivity of PCR, up to 30–40% of suspected cases of enteroviral meningitis elude specific diagnosis [Rotbart, 1995a].

Because human transmission of EV is mainly by the fecal-oral route [Rotbart, 1995b], the evaluation of pharyngeal swabs in children presenting with aseptic meningitis may enhance specific diagnosis. Previous studies report that combined stool and CSF findings [Glimaker et al., 1992], urine and CSF findings [Nielsen et al., 1996], or pharyngeal swabs and CSF findings [Andréoletti et al., 1998; Rotbart et al., 1997] increase the detection of EV, but none of those studies attempted to improve the diagnosis of aseptic meningitis due to EV or evaluated the specificity of EV detection in a non-CSF specimen. Nevertheless, Rotbart and Romero [1995] stated that a positive PCR detection in throat swabs alone should be considered as nonsignificant. The prospective study undertaken examined combined upper respiratory tract (URT) and CSF findings for the diagnosis of EV aseptic meningitis and attempted to determine the specificity of URT findings in patients with negative findings in the CSF.

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## MATERIALS AND METHODS

In a prospective study that was carried out during a 4-month period (1 June 1996 to 1 October 1996), pharyngeal swabs and CSF samples were taken from children who were hospitalized with aseptic meningitis. During the same period, a control population of age- and sex-matched control children without meningitis had pharyngeal samples taken to determine the prevalence of asymptomatic pharyngeal carriage of EV. Children of both groups were enrolled by obtaining informed consent from their parents.

Twenty-eight children (age range: 10 days to 17 years) were included. Each patient that presented with clinical signs and symptoms of meningitis (inclusion criteria: fever >38.5°C, headaches, photophobia, neck stiffness, and vomiting) was included in the test group (cases 1–28). CSF samples were lacking in patients 1–3. The control group (cases 29–56) had pharyngeal swabs taken on the day of admission to the hospital but no CSF sample.

CSF samples were aliquoted into three different tubes and then sent to the laboratory. By using standard methods, PCR for EV, herpes simplex, and mumps virus was carried out on tube 1 [Boriskin et al., 1993; Lakeman et al., 1995; Leparc et al., 1994]. The enterovirus PCR assay detects all EV serotypes except for EV 22 and EV 23. Rhinoviruses are not detected with this assay.

When sufficient volume was obtained, the CSF was also inoculated onto cell lines (MRC5, VERO, BGM, and HEP2) for viral culture as described previously [Lina et al., 1996]. The second tube was used for cytological and chemical tests, and the third tube was used for bacterial detection and culture. Briefly, CSF samples were streaked onto chocolate agar plates with polyvitex enrichment mixture prior to incubation for 48 hours at 37°C under 5% $CO_2$ and onto trypticase soy agar plates containing 5% sheep blood (bioMérieux, Marcy l'Etoile, France) that were incubated at 37°C. CSF samples were also inoculated into brain-heart broth supplemented with 10% (volume/volume) blood cell extract (Sanofi Diagnostic Pasteur, Marnes la Coquette, France). Antigen detection was carried out systematically for *Streptococcus pneumoniae*; *Haemophilus influenza*; *Nesseiria meningitidis* A, B, and C; *Streptococcus* B; and *Escherichia coli* K1 by using the Pastorex kit (Sanofi Diagnostic Pasteur).

Pharyngeal swabs from both the test and control groups were processed identically. They were placed in fetal calf serum free-transport medium and sent immediately to the laboratory. The samples were then inoculated onto tissue cell culture (MRC5, VERO, BGM, and HEP2) and tested for mumps and EV by using reverse transcriptase RT-PCR. Statistical analysis was performed by using a $X^2$ test.

## RESULTS

The male:female ratio and the median age were similar in both groups (Table I). CSF samples in the test group had a median white blood cell count of 187 cells per $mm^3$ (range, 2–1,560), CSF protein was >0.5 g per liter in only eight cases (median, 0.41; range, 1.06–0.12 g per liter), and CSF glucose was normal in all cases (median, 3.22; range, 1.97–3.95 g per liter). The differential count of white blood cells in CSF was not recorded. Bacterial detection and culture were negative in all cases.

Among the 25 CSF samples that were processed for virologic tests, nine were positive for viral detection (36%), including eight EV (32%) and one herpes simplex virus (4%). No mumps virus was detected. All but one virus were detected only by using PCR.

In patients from the test group, EV was detected in pharyngeal samples from 17 patients (60.7%): Of these, 15 were determined in tissue cell cultures (including seven Echo virus 30, four Coxsackie virus B5, two Coxsackie virus A24, one Echo virus 11, and one Echo virus 7), and 12 were determined by using PCR (Table I). Two cases were positive only by RT-PCR, and five cases were only culture positive. The remaining negative samples were also negative for mumps virus by using PCR.

In control patients, five pharyngeal samples were positive by RT-PCR (17.8%), No viruses were detected with tissue cell cultures. Mumps virus was not detected by using PCR. The difference of positive pharyngeal samples between test and control patients was statistically significant ($P < 0.001$).

## DISCUSSION

The male:female sex ratio of 3:6 that was observed in this study confirmed previous reports suggesting that EV meningitis is more common in male children [Khalfan et al., 1998; McIntyre and Keen,. 1993]. The combination of pharyngeal and CSF findings increased the detection of viruses from 36% to 71.5%. The only viruses that were detected in pharyngeal samples were EV viruses. Those samples were positive in 17 of 28 (60.7%) patients, giving positive results in eight patients cases with negative CSF (Table I). Among the 17 positive nasopharyngeal swabs, ten (72%) were positive by both PCR and culture, two were positive by PCR, and five were positive only by culture. These discrepancies have been reported in previous studies [Andréoletti et al., 1998; Rotbart et al., 1997]. They may be due to the presence of neutralizing antibodies on the surface of the viruses, to nontissue cell culture-adapted EVs for those only detected by PCR, or to the presence of PCR inhibitors in the samples (e.g., bacterial proteases) when EVs were detected only by culture. Six patients had both CSF and pharyngeal samples that were positive for EV, and only two patients had positive CSF and negative pharyngeal samples. Of the patients with aseptic meningitis, 21 in 28 (75%) had viral detection by CSF and/or pharyngeal analysis. All patients with multiple positive findings had consistent results. Five control patients (17.8%) had pharyngeal swabs that were positive for EV by PCR only. The lack of cultivable viruses was probably due either to low inoculum size or

TABLE I. Detection of Viruses and Characteristics in Both Patients (1–28) and Controls (29–56)

| Case no. | Samples[a] | | | | | Age | Sex | Cause of hospitalisation |
|---|---|---|---|---|---|---|---|---|
| | CSF | | | Pharyngeal swabs | | | | |
| | PCR HSV | PCR EV | Culture | PCR EV | Culture | | | |
| 1 | na | na | na | + | Cox B5 | 4 y | F | Aseptic meningitis |
| 2 | na | na | na | + | Cox A24 | 6 y | M | Aseptic meningitis |
| 3 | na | na | na | + | Echo 30 | 11 y | M | Aseptic meningitis |
| 4 | – | – | – | – | – | 11 y | F | Aseptic meningitis |
| 5 | – | – | – | – | – | 6 y | M | Aseptic meningitis |
| 6 | – | – | – | – | Cox A24 | 12 y | M | Aseptic meningitis |
| 7 | + | – | – | – | – | 1 m | M | Aseptic meningitis |
| 8 | – | + | – | + | Cox B5 | 1 m | M | Aseptic meningitis |
| 9 | – | + | Echo 30 | + | Echo 30 | 4 y | M | Aseptic meningitis |
| 10 | – | – | – | – | Echo 30 | 5 y | M | Aseptic meningitis |
| 11 | – | – | – | + | Echo 30 | 6 y | F | Aseptic meningitis |
| 12 | – | – | – | + | Echo 7 | 2 m | M | Aseptic meningitis |
| 13 | – | – | – | – | – | 10 y | M | Aseptic meningitis |
| 14 | – | – | – | – | – | 1 m | M | Aseptic meningitis |
| 15 | – | + | – | – | – | 6 y | M | Aseptic meningitis |
| 16 | – | – | – | – | – | 9 y | M | Aseptic meningitis |
| 17 | – | + | – | + | Cox B5 | 4 m | M | Aseptic meningitis |
| 18 | – | + | – | – | Cox B5 | 8 y | M | Aseptic meningitis |
| 19 | – | – | – | – | Echo 30 | 7 y | F | Aseptic meningitis |
| 20 | – | – | – | + | Echo 11 | 1 m | M | Aseptic meningitis |
| 21 | – | – | – | – | – | 1 m | M | Aseptic meningitis |
| 22 | – | – | – | – | – | 5 m | M | Aseptic meningitis |
| 23 | – | + | – | + | Echo 30 | 17 y | F | Aseptic meningitis |
| 24 | – | + | na | – | – | 5 y | F | Aseptic meningitis |
| 25 | – | – | – | – | – | 11 d | M | Aseptic meningitis |
| 26 | – | + | – | + | – | 1 y | M | Aseptic meningitis |
| 27 | – | – | – | + | – | 11 y | M | Aseptic meningitis |
| 28 | – | – | – | – | Echo 30 | 12 y | M | Aseptic meningitis |
| 29 | na | na | na | + | – | 4 y | F | Asthma |
| 30 | na | na | na | – | – | 6 y | M | Orthopaedic disorder |
| 31 | na | na | na | – | – | 14 y | M | Cranial traumatism |
| 32 | na | na | na | – | – | 11 y | F | Paludism |
| 33 | na | na | na | – | – | 7 y | M | Fever |
| 34 | na | na | na | – | – | 13 y | F | Tetanoid presentation |
| 35 | na | na | na | – | – | 8 m | M | Down's syndrome |
| 36 | na | na | na | – | – | 1 y | F | Sudden unexpected death |
| 37 | na | na | na | – | – | 1 y | M | Pharyngitis |
| 38 | na | na | na | + | – | 5 y | M | Asthma |
| 39 | na | na | na | – | – | 8 y | F | Car accident |
| 40 | na | na | na | – | – | 3 m | M | Renal failure |
| 41 | na | na | na | + | – | 9 y | M | Fever |
| 42 | na | na | na | – | – | 1 m | M | Enterocolitis |
| 43 | na | na | na | – | – | 7 y | M | Abdominal pain |
| 44 | na | na | na | – | – | 9 y | M | Crohn disease |
| 45 | na | na | na | – | – | 8 m | M | Suspicion of herpes |
| 46 | na | na | na | – | – | 7 y | M | Pharyngitis |
| 47 | na | na | na | – | – | 7 y | F | Orthopaedic disorder |
| 48 | na | na | na | – | – | 1 m | M | Fever |
| 49 | na | na | na | – | – | 1 m | F | Suspicion of infectious disease |
| 50 | na | na | na | + | – | 7 m | M | Sudden unexpected death |
| 51 | na | na | na | – | – | 16 y | M | Myelin disorder |
| 52 | na | na | na | – | – | 2 y | F | Neutropenia |
| 53 | na | na | na | – | – | 10 d | M | Septicemia |
| 54 | na | na | na | + | – | 1 y | M | Leukemia and fever |
| 55 | na | na | na | – | – | 10 y | M | Renal disease |
| 56 | na | na | na | – | – | 12 y | M | Car accident |

[a]CSF, cerebrospinal fluid samples; na, not available; +, positive; –, negative; M, male; F, female; y, year; m, month; d, days; HSV, herpes simplex virus; EV, enterovirus; Cox, Coxsackie virus; Echo, Echovirus. Mumps reverse transcriptase-polymerase chain reaction (RT-PCR) performed in CSF and pharyngeal samples was negative in all cases; no CSF was sampled in control subjects. Test group, male:female ratio, 3.6, median age, 5.22 (range 11 days to 17 years); control group, male:female ratio, 2.5, median age, 5.25 (range 10 days to 16 years).

to the presence of large amounts of neutralizing antibodies on the viral surface that prevent cell line propagation. However, the rate of detection of EV from pharyngeal samples was significantly less ($P < 0.001$) in the control group versus the test patients. Detection of EV in the control group may reflect either an asymptomatic carriage or an ongoing infection. Analysis of those five cases showed that two patients presented

with asthma, two patients presented with fever of unknown origin at admission (including one patient with leukemia), and one patient was an infant who had a sudden, unexpected death. The latter presentation has been reported as a consequence of disseminated EV infection in neonates [Grangeot-Keros et al., 1996]. The fever observed in two patients could also be related to an ongoing EV infection with no central nervous system disease. These samples that were only positive by PCR confirm the analysis by Rotbart and Romero [1995] of such results: EV detection by PCR in pharyngeal samples is not pathognomonic of aseptic meningitis. In our study, the positive predictive value was 77%. When analyzing results of tissue cell cultures, the sensitivity was 53.5%, with a positive predictive value of 100% and a negative predictive value of 68%. URT findings using tissue cell culture statistically enhance the detection rate compared with CSF examination alone ($P < 0.05$).

One concern in the current study was the low detection rate by using PCR examination of CSF (36% positive). In previous studies, this technique was shown to be very sensitive [Beaulieux et al., 1997], reaching 50% positive in CSF sampled during an epidemic [Khalfan et al., 1998]. Previously, we also evaluated the putative inhibitor effect of hemoglobin in CSF samples, showing that it did not inhibit EV detection [Lina et al., 1996]. The low detection rate, therefore, might be due to three factors: the low inoculum size of EV in CSF, infection with EV serotypes in which the genomic sequences yield partial malaligment with the primers, and a delay in CSF collection when admission to the hospital was not at the onset of the disease. Viral shedding during enterovirus infection is known to persist in the respiratory tract beyond viral presence in CSF. During the study, each patient had a CSF sample taken during admission in order to prevent a false negative result due to delayed samples. Concerning the sensitivity of the PCR assay, previous work has shown that the PCR used can be adapted for the detection of all but two EV serotypes [Leparc et al., 1994], but it is likely that its sensitivity varies with the serotype. Nevertheless, PCR remains more sensitive than conventional tissue cell culture for EV detection in CSF [Lina et al., 1996].

Several studies have attempted to improve rapid EV neurological infection by combining findings from different samples, but none evaluated the specificity of findings in non-CSF samples for the diagnosis of aseptic meningitis. Other than potential healthy carriers, because of recent infection, some EV excretor children may be found positive nonspecifically for EV. In this case, the rate of nonspecific detection is correlated directly with the duration of the shedding from each potential excretor site. It was decided to evaluate pharyngeal detection because of its relatively low duration of shedding (2 weeks) compared with shedding in feces and because it was easy to perform. The comparison of URT findings in the two groups from the study confirmed that the detection of EV by tissue cell culture in URT samples of children presenting with aseptic meningitis may be relevant, giving highly specific, positive results in meningitis cases, with a positive predictive value of 100% and a negative predictive value of 74%.

It is concluded that this combination of findings is helpful for determining the etiologic agent of aseptic meningitis. Combined with hematologic and biochemical tests that suggest viral infection, this diagnostic scheme may be of help in reducing hospitalization and avoiding unnecessary treatment. These findings need to be confirmed by a larger multicenter study.

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